diagenode

Innovating Epigenetic Solutions

AUTO HISTONE ChIP-seq KIT

Auto Histone ChIP-seq kit protein A x16
Cat. No. C01010020;
(Old Cat. No. AB-Auto02-A016)

Auto Histone ChIP-seq kit protein G x16
Cat. No. C01010021;
(Old Cat. No. AB-Auto02-G016)

Auto Histone ChIP-seq kit protein A x100
Cat. No. C01010022;
(Old Cat. No. AB-Auto02-A100)

Auto Histone ChIP-seq kit protein G x100
Cat. No. C01010023;
(Old Cat. No. AB-Auto02-G100)

Version 5 I 21.01.14

Contents

Introduction 4
SX-8G IP-Star Automated System for ChIP, MeDIP &MBD 5
Kit Method Overview 8
Kit Materials 9
Kit Content 9
Required Materials Not provided 10
How to perform Automated ChIP in the SX-8G IP-Star® Compact 11
Chromatin preparation 12
Running a protocol 13
How to perform Automated ChIP in the SX-8G IP-Star® 20
Chromatin preparation 21
Reagents dispensing 22
Running a protocol 24
Quantitative PCR & Data analysis 28
Results 30
Additional protocol 31
Troubleshooting Guide 33
Technical Assistance 34
Ordering Information . . . . Back Cover

Introduction

The Diagenode SX-8G IP-Star® Automated System automates immunoprecipitation and increases reproducibility

Diagenode, the leading provider of complete solutions for epigenetics research, offers a variety of end-to-end systems to streamline DNA methylation and chromatin immunoprecipitation workflows. Central to this full offering is Diagenode's Automated Systems, simple yet robust automated bench-top instruments that standardize different epigenetic applications (i.e. ChIP, MeDIP or MethylCap). Diagenode designed these automation systems to make ChIP and DNA methylation studies accessible and reproducible, and ensure consistent data in every experiment.

Diagenode Automated Systems will produce consistent results from any operator regardless of the day, the experimental run, or the lab. Robust and reproducible results is a major goal of today's high resolution epigenomic studies.

Diagenode Automated Platforms replace the numerous manual, error-prone steps of complex epigenetic applications with a reliable, highly consistent and automated process that requires minimal operator intervention. We empower researchers to simplify the tedious protocols and the complexity of many epigenetic protocols. In addition, Diagenode Automated Systems minimize sample carryover, data variability, and costly errors. The platforms offer full workflow support for epigenetics research, utilizing our complete kits and laboratory-validated protocols to rapidly deliver high-quality and consistent data.

Auto Histone ChIP-seq kit

The Auto Histone ChIP-seq kit was developed to enhance the utility of the ChIP procedure, allowing one to perform many more ChIPs per day and per week. The entire procedure can be performed in a single day, since two overnight incubations have been eliminated. The IP has been optimized to specifically select and precipitate the chromatin with the use of our validated antibodies, buffers and protocols. Furthermore, the use of our automated system will drastically increase the consistency of your ChIP assay.

The Auto Histone ChIP-seq kit allows quick and highly specific chromatin IP sample analysis. The Auto ChIP kit protocol has been improved to allow researchers to work with smaller volumes than other traditionally used methods. The kit ensures the use of small amounts of reagents per reaction (including antibodies and buffers) and also provides you with fewer buffers in comparison with other kits.

The Auto Histone ChIP-seq Kit has been validated to perform ChIP-seq experiments using antibodies directed against histone modifications. The combination of this high quality kit and the IP-Star allows Chromatin IP to be performed in less than 10 hours. Starting with sheared chromatin, the Automated System provides purified immunoprecipitated DNA from your sample. The Auto ChIP kit protocol has been validated using chromatin sheared by sonication using the Bioruptor.

Customer Feedback

Not only does the IP-Star eliminate the problem of human variation associated with producing our samples, it also enables us to produce 1000-2000 ChIP-seq samples per year very reliably. The IP-Star reduces our processing time down from one day of manual work to just one overnight run with only 30 minutes of hands-on work. The IP-Star has made all our ChIPs consistent and the process completely reliable regardless of the operator or the time of day.

Dr. John Lambourne, Postdoctorate Researcher at the Innovation Centre, McGill University, Canada.

SX-8G IP-Star® and SX-8G IP-Star® Compact Systems for automation of epigenetic applications

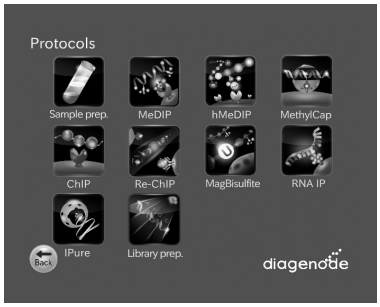
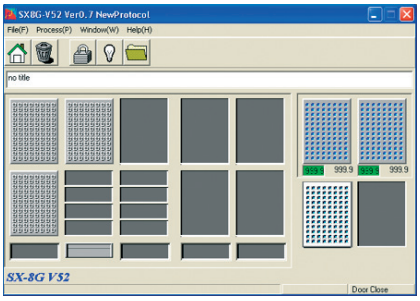
Diagenode has developed two automated platforms (SX-8G IP-Star® and SX-8G IP-Star® Compact) designed to increase your lab's productivity, efficiency and experimental reproducibility. The two automated platforms are capable of processing up to 16 samples per cycle. The automated systems processes sheared chromatin (or DNA) to deliver purified DNA ready for qPCR, amplification, microarray and sequencing analysis. Both, the SX-8G IP-Star® and SX-8G IP-star® Compact have an easy-to-use open software that provides you with flexibility. This allows you to create your personal protocol according to your specific needs.

Major benefits of Diagenode Automated Platforms

SX-8G IP-Star® Compact

Sx-8G IP-Star®

- High resolution ChIP-seq and MeDIP-seq profiles
- Automated library preparation for Next Generation sequencing
- Reduces hands on time to just 30 minutes
- Reduces variability between operators and labs
- Ideal for low sample starting amounts
- Compatible with Diagenode Kits (Auto ChIP kit, Auto Histone ChIP-seq kit, Auto Histone ChIP-seq kit, Auto MeDIP kit, Auto MethylCap kit, Auto hMeDIP, Auto IPure kit)
- Reduces cross-contamination

	SX-8G IP-Star® Compact	SX-8G IP-Star®
Applications	**ChIP-seq, MeDIP-seq, MethylCap-seq, hMeDIP, IPure, Sample preparation, Re-ChIP, MagBisulfite, RNA-IP, Library preparation for NGS platforms.**	**ChIP-seq, MeDIP-seq, MethylCap-seq, hMeDIP, IPure, Sample preparation, Re-ChIP, MagBisulfite, RNA-IP.**
Software		
User interface	Intuitive touch screen panel	PC Software
User friendly	Software training not required	Software training before use
Dispensing	Automated dispension of assay reagents	Manual dispension of assay reagents
Protocol optimization (flexible parameters)	Antibody coating (temperature, time, mixing speed) Immunoprecipitation (temperature, time, mixing speed) Washes (temperature, time, mixing speed)	Antibody coating (temperature, time) Immunoprecipitation (temperature, time)
Characteristics	750W x 740 D x 610 H | 100 kg 8 Nozzles X-Y-Z axis | 4 – 95°C	1070W x 650 D x 780 H | 130 kg 8 Nozzles X-Y-Z axis | 4-95°C

Improved reproducibility

Our SX-8G IP-Star will increase the immunoprecipitation reproducibility between IPs performed by the same as well as by different operators (see figure 1 and 2 below). Reagents (Antibodies, buffers,...) and sheared chromatin were identical for "ManChIP" and "AutoChIP". The SX-8G IP-Star Automated system removes variation that can be created by manual handling and allows you to optimize and standardize your assay within a lab. The SX-8G IP-Star is designed to improve the accuracy and the reproducibility of any immunoprecipitiation experiment.

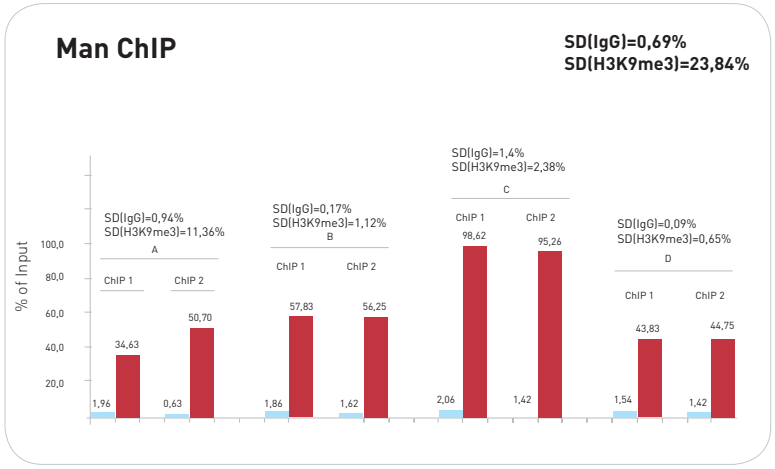


Figure 1: Manual ChIP. Four different operators have each performed two ChIP experiments using H3K9me3 antibody on the genomic region SAT2 (positive locus). 10,000 Hela cells have been used per IP. Reagents and sheared chromatin were identical per assay. The standard deviations between the ChIPs performed by the same operator and between the four different operators are displayed.

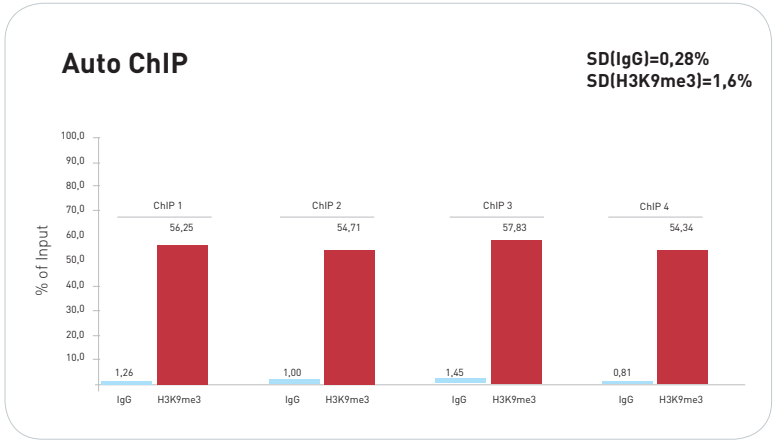


Figure 2: Automated ChIP. Four ChIP experiments using H3K9me3 antibody on the genomic region SAT2 (positive locus) have been performed by the SX-8G IP-Star. 10,000 Hela cells have been used per IP. Reagents and sheared chromatin were identical per assay. The standard deviations between the four ChIPs performed by the SX-8G IP-Star are displayed.

Kit Method Overview

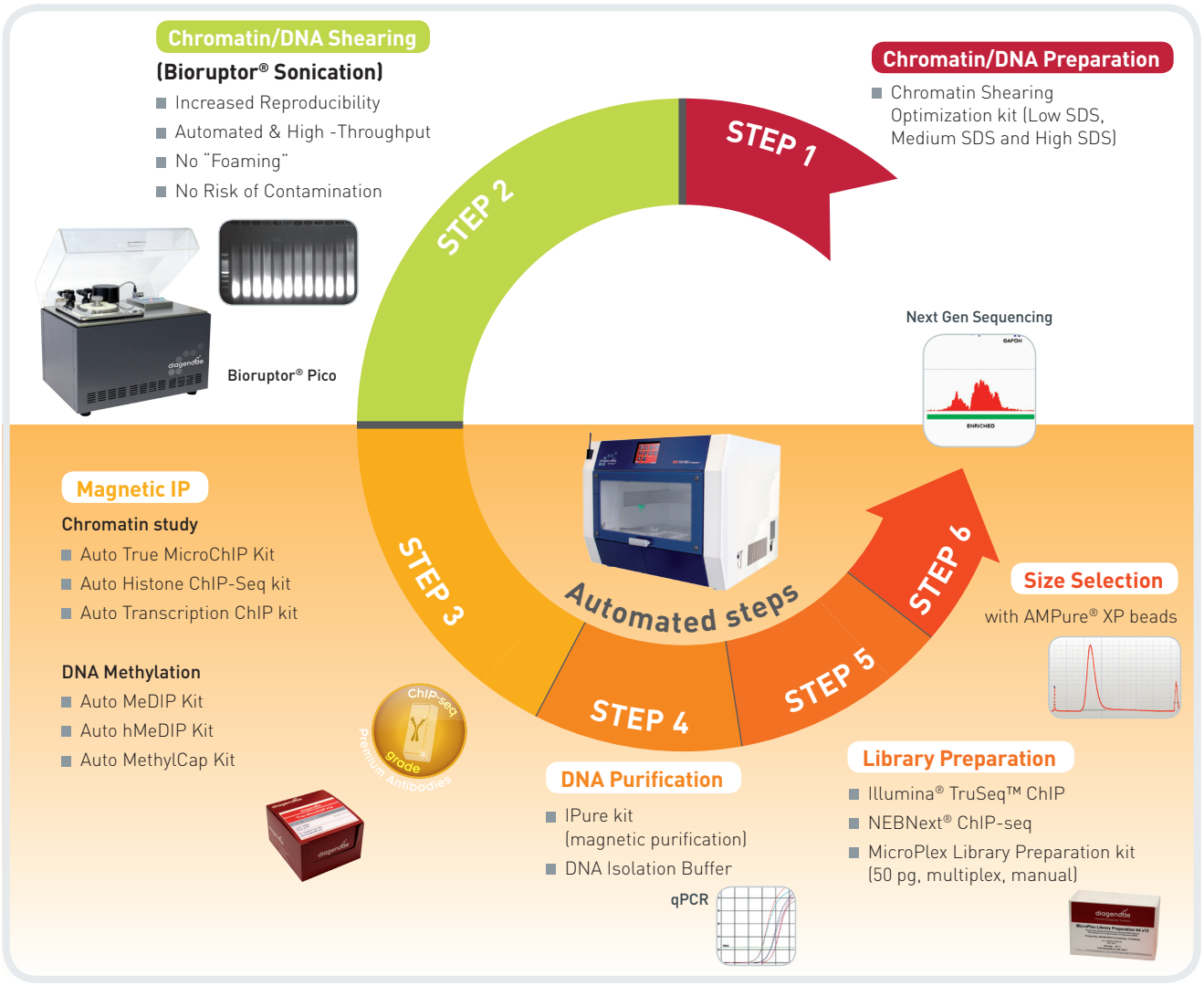


Figure 3. Diagenode provides a full suite of automated solutions for ChIP experiments.

For Step 1, we offer products to isolate nuclei and chromatin. Step 2 describes reproducible sample shearing with the Bioruptor® product line. In Step 3 and Step 4, the Diagenode IP-Star Compact provides error-free, walk-away automation for all your immunoprecipitation and antibody capture needs.

Kit Materials

Kit Content

The Auto Histone ChIP-seq kits contains reagents to perform 16 or 100 Chromatin Immunoprecipitations by using the SX-8G IP-Star Automated System. The kit content is described in Table 1. Upon receipt, store the components at the temperatures indicated in Table 1.

Note: The kit has been designed to work with histone antibodies and allows to perform ChIP experiments starting with sheared chromatin.

Table 1. Kit content

Description	Quantity (x16)	Quantity (x100)	Storage
1.25 M Glycine	2 ml	15 ml	4°C
Protein G or Protein A coated magnetic beads	220 µl	1500 µl	4°C
ChIP-Buffer H	20 ml	125 ml	4°C
Rabbit IgG or Mouse IgG	15 µl	110 µl	4°C
Protease inhibitor mix (200x)	100 µl	700 µl	- 20°C
5% BSA (10x solution)	40 µl	200 µl	- 20°C
1 M Sodium Butyrate	40 µl	200 µl	- 20°C
Wash Buffer H1	4 ml	30 ml	4°C
Wash Buffer H2	4 ml	30 ml	4°C
Wash Buffer H3	4 ml	30 ml	4°C
Wash Buffer H4	4 ml	30 ml	4°C
Elution Buffer H	4 ml	30 ml	4°C
5 M NaCl	220 µl	1500 µl	4°C
Water	2 ml	10 ml	4°C
DNA Isolation Buffer (DIB)	4 ml	30 ml	4°C
Proteinase K	40 µl	220 µl	-20°C

Table 2. Reagents available separately

Description	Reference	Description	Quantity	Storage
1 M Sodium butyrate	C12020010	-	1 ml	-20°C
Protein A-coated paramagnetic beads	C03010020-220 C03010020-660 C03010020-150	The beads are supplied in solution with; detergent and 0.02% sodium azide.	220 µl 660 µl 1500 µl	4°C Do not freeze
Protein G-coated paramagnetic beads	C03010021-220 C03010021-660 C03010021-150	The beads are supplied in solution with; detergent and 0.02% sodium azide.	220 µl 660 µl 1500 µl	4°C Do not freeze
Rabbit IgG	C15410206	1 µg/µl	250 µl	4°C
Mouse IgG	C15200001	1 µg/µl	15 µl	4°C
Antibodies	-	-	www.diagenode.com	
Primer pairs	-	5µM each (Rv & Fw)	www.diagenode.com	

Table 3. Kits and Modules available separately

Description	Reference	Quantity
Chromatin shearing optimization kit - Low SDS	C01020010	1 kit
Chromatin shearing optimization kit - Medium SDS	C01020011	1 kit
Chromatin shearing optimization kit - High SDS	C01020012	1 kit
Auto IPure	C02010012	100 rxns

Table 4. Plastics and consumables available separately

Description	Reference	Quantity
200 µl tube strips (12 tubes/strip) + cap strips	C30020001	80
200 µl tube strips (8 tubes/strip) + cap strips for SX-8G IP-Star® Compact	C30020002	120
96 well microplates for IP-Star®	C30080030	10
Tips (box)	C30040021	960
Tips (bulk)	C30040020	1000
2 ml microtube for SX-8G IP-Star® Compact	C30010014	100
Large reagent container for SX-8G IP-Star® Compact	C30020004	20
Medium reagent container for SX-8G IP-Star® Compact	C30020003	10

Required Materials Not Provided

Reagents

- Gloves to wear at all steps
- Phosphate buffered saline (PBS)
- Trypsin-EDTA
- Formaldehyde (fresh MolBiol Grade)
- qPCR reagents
- Quant-IT dsDNA HS assay kit (Invitrogen)

Equipment and accessories

- DiaMag02 magnetic rack (Cat. No. B04000001)
- Cell counter
- BioruptorR sonication apparatus
- Diagenode 1.5 ml TPX microtubes (optimized for chromatin shearing with Bioruptor) (Cat. No. ZC30010003/ ZC30010004)
- Centrifuge for 1.5 ml tube
- Vortex
- Qubit system
- qPCR cycler

How to perform Automated ChIP in the SX-8G IP-Star® Compact

SX-8G IP-STAR® COMPACT

How to perform Automated ChIP in the SX-8G IP-Star® Compact

Chromatin preparation

STEP 1. Cell collection and DNA-protein crosslinking

1. Collect the cells by trypsinisation and wash two times with PBS.
2. Count the cells and resuspend them in PBS to obtain up to 10 million cells in 500 µl of PBS. Aliquot 500 µl of cell suspension in 1.5 ml tubes.
3. Add 13.5 µl of 36.5% formaldehyde per 500 µl sample. Mix by gentle vortexing and incubate for 8 minutes at room temperature to allow fixation to take place.
4. Stop the fixation by adding 57 µl of Glycine solution. Mix by gentle vortexing and incubate for 5 minutes at room temperature. Work on ice from this point onwards.
5. Centrifuge at 1,600 rpm (250 x g) for 5 minutes at 4°C and gently aspirate the supernatant without disturbing the cell pellet.
6. Wash the cells twice with 1 ml PBS.

STEP 2. Cell lysis and chromatin shearing

We recommend the use of Diagenode Bioruptor devices in combination with our shearing optimization kits (Table 3. p.10) for the preparation of the sheared chromatin.

STEP 3. Sheared chromatin and beads preparation

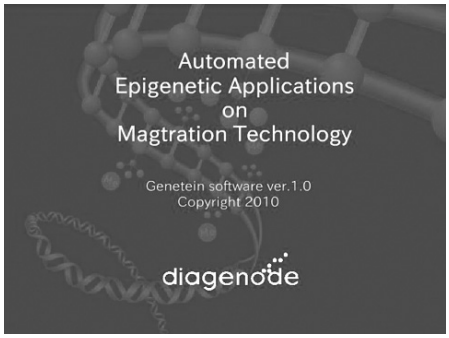
1. Start with sheared chromatin in Low, Medium or High SDS concentration depending on the cell type and the amount of cells sheared.
2. Dilute your sheared chromatin with ChIP Buffer H to reach a final concentration of 0.1-0.15% SDS. Dilute 10 times if using Chromatin shearing optimization kit - High SDS and 5 times if using Chromatin shearing optimization kit - Medium SDS.
3. Protease Inhibitors are provided in the kit. Add to the diluted sheared chromatin, protease inhibitors to a final concentration of 1X

Note 1: please mind in advance about the cell concentration during shearing process as the working IP volumes in the automated systems are 100 µl and 200 µl.

Note 2: In general, Diagenode does not recommend to preblocked Diagenode magnetic beads. BSA is however provided in this kit for customers that would like to proceed with preblocking or to add BSA in the immunoprecipitation reaction. For preblocking proceed as follow:

1. Pippette 100 ul of beads. Add 390 ul CHIP Buffer H and 10 ul BSA 5% (0.1% final concentration)
2. Incubate 60 minutes by rotation
3. Washed once with 100 ul of ChIP BUffer H.
4. Resuspend your beads in 100 ul ChIP Buffer H.
   10 ul of magnetic beads will bind up to 2.5 ug of Antibody.

Running a protocol



Diagenode Splash Screen – A0

After the software start-up screen disappears, the Diagenode splash screen is displayed for several seconds, and then disappears.

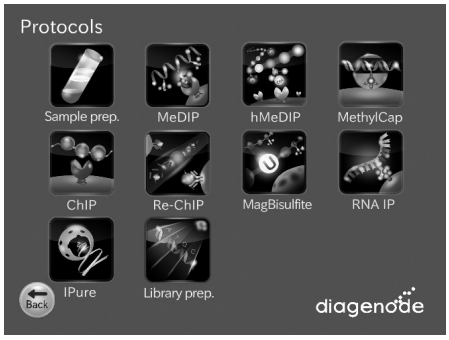
Start Screen – Top menu

After the Digenode splash screen disappears, the start screen is displayed. This is the first active window; it allows the user to enter into three different parts of the software.

USER ACTIONS:

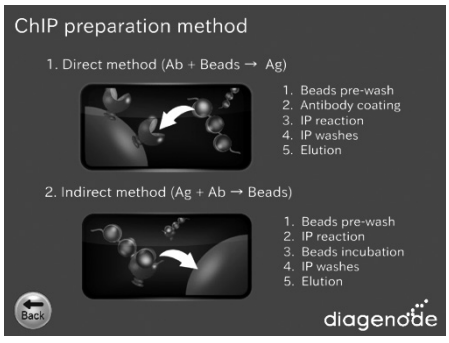
Buttons:

- Protocols
- Maintenance (for technical service)
- Information (Diagenode contact details)



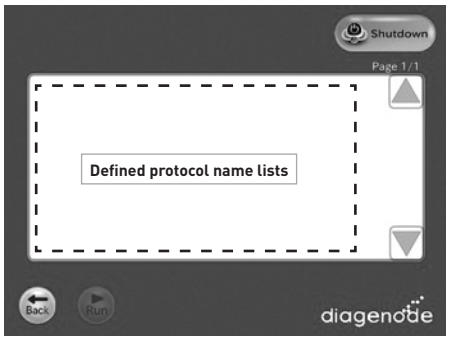
Protocols screen

All available protocols are displayed on this screen.



Screen – [ChIP preparation methods]

The user can select between protocols for direct or indirect ChIP methods.

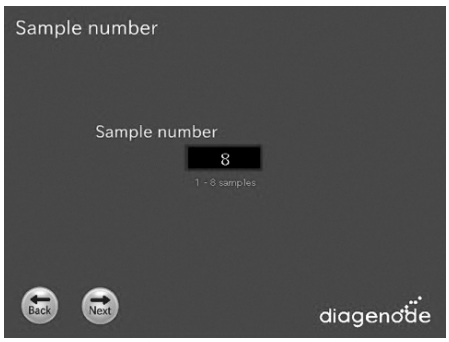


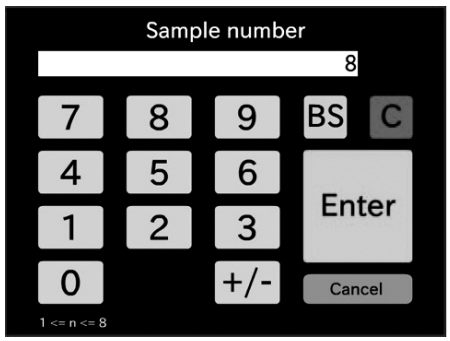
Screen – [Categories Name] Protocol List

After the user presses the "[Categories Name]" button, the "[Categories Name]" appears. When selected the protocol on the protocol list, the "Run" button shall turn executable.

Buttons:

- The user presses the "Back" button. The user returns to the "Protocols" screen.
- The user presses the "Shutdown" button. The screen shall be changed to "Power Off".
- The user presses the "Run" button. The screen shall be changed to "Sample number".
- ▲ Page up the list box.
- ▼ Page down the list box





Keyboard

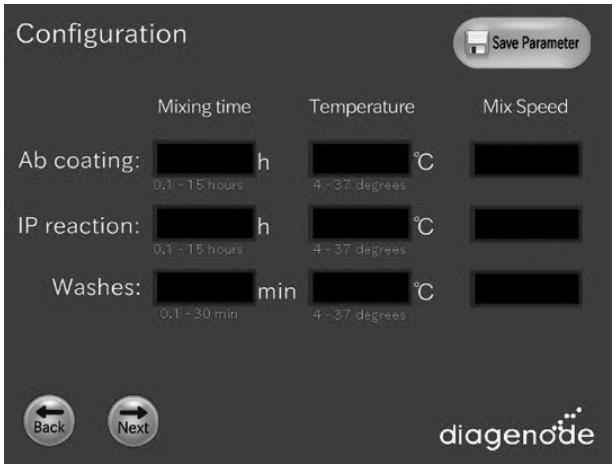
Screen – Sample number

After the user presses the "Run" button, the "Sample number" appears.

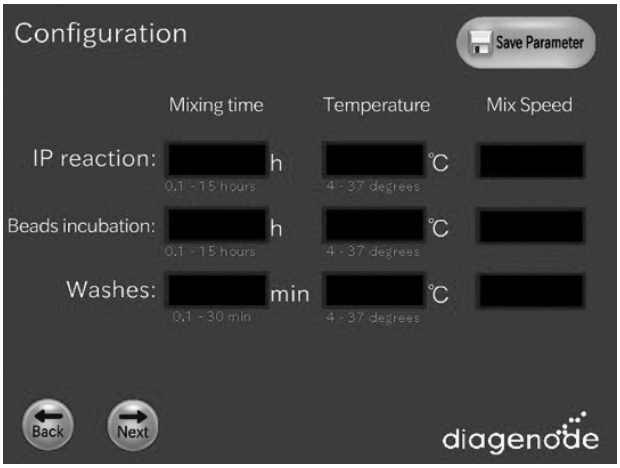
Buttons:

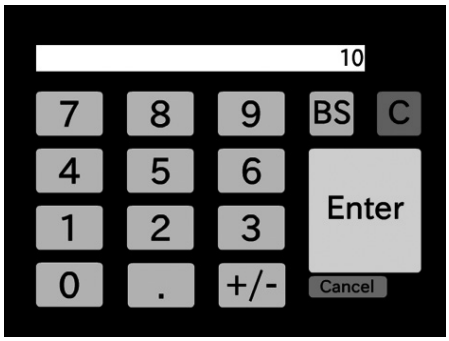
- The user presses the "Sample number" Text box. Then screen will be changed to keyboard.
- The user presses the "Back" button. The user returns to the "Protocol List" screen.
- The user presses the "Next" button. Then screen shall be changed to "Configuration" or "Layout information".

DIRECT ChIP

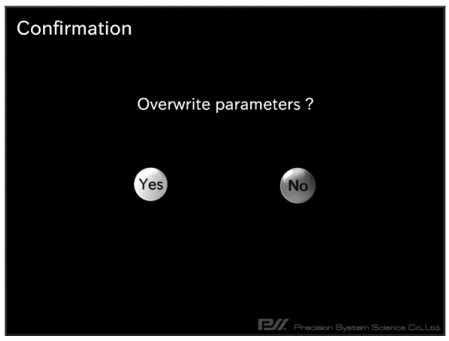


INDIRECT ChIP





Keyboard

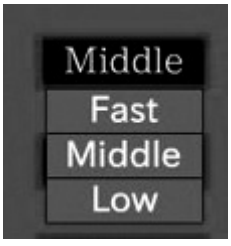


Screen – Configuration

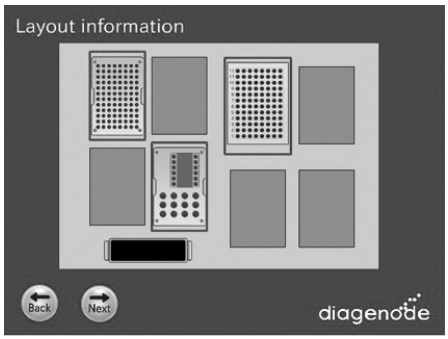
After the user presses the next button from the "Sample number" screen, the "Configuration" screen appears.

Buttons:

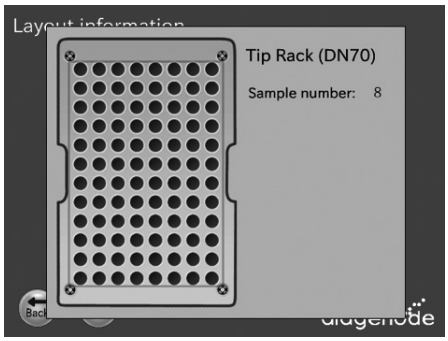
- The user presses the "Back" button. The user returns to the "Protocol List" screen.
- The user presses the "Next" button. The screen shall be changed to "Layout information".
- The user presses the "Save Parameter" button. The screen will be changed to "Save Parameter - Confirmation".
  - OK – Current parameters shown in the Display View will be stored to the [Protocol].ptd. And, returns the user to the display of the "Configuration" screen.
  - No – Returns the user to the display of the "Configuration" screen.
- The user presses the Text box. The screen will be changed to Keyboard or Speed list menu.



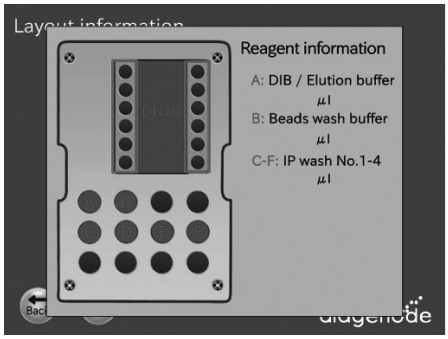
Speed list menu



Layout information



Block-Tip



Block-Regent Tip Rack

Screen – Layout Information

After the user presses the "next" button from "Sample number" screen or "Configuration" screen, the "Layout Information" screen appears.

Buttons:

- The user presses the "Back" button. The user returns to the previous screen.
- The user presses the "Next" button. The screen shall be changed to "Set confirmation".
- When the user presses a block, that block is magnified on the work surface layout background. The magnified view provides a better display of the correct method setup for that block on the work surface.
- Based on the selected protocol, the user follows the indications provided in the screens to set up correctly the different reagents and samples.

Screen – Layout Information

Beads Wash Buffer: ChIP Buffer H

BID/Elution buffer: DIB or Elution Buffer

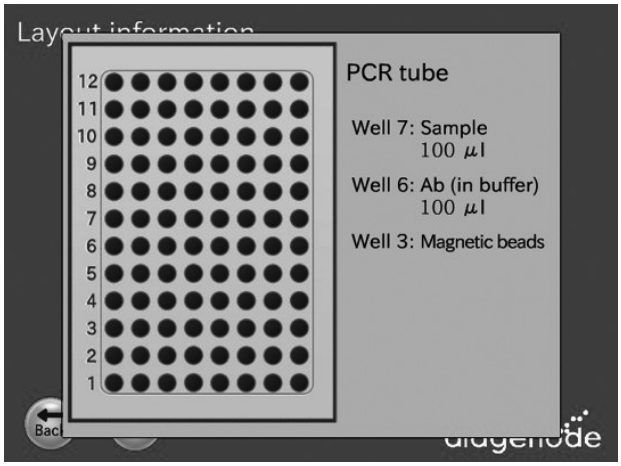
IP wash 1: Wash Buffer H1

IP Wash 2: Wash Buffer H2

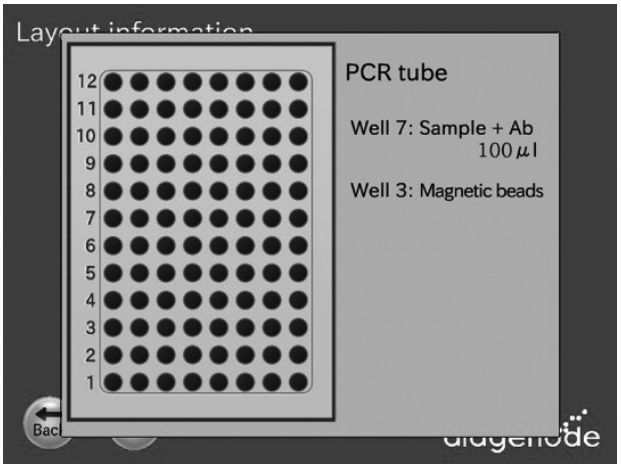
IP wash 3: Wash Buffer H3

IP wash 4: Wash Buffer H4

DIRECT ChIP



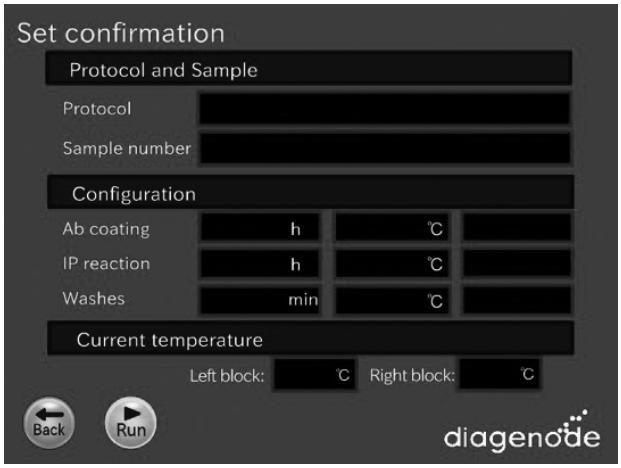
INDIRECT ChIP



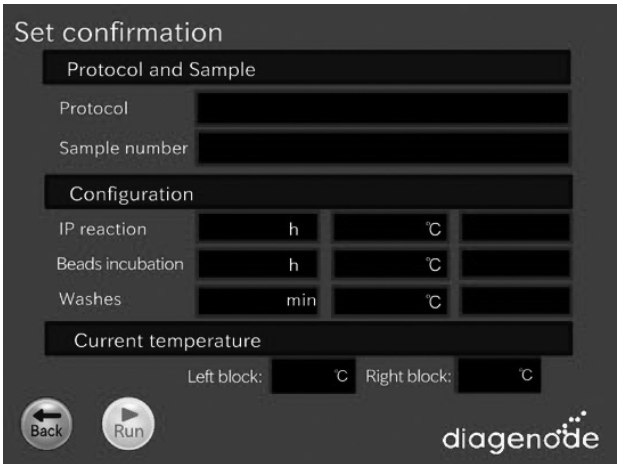
NOTE 1: If required, add 2 ul of BSA (5%) to the chromatin sample when running ChIP-100 ul protocols or 4 ul of BSA (5%) to the chromatin sample when running ChIP-200 ul protocols

NOTE 2: If required, NaBu (HDAC inhibitior, 20mM final concentration) or other inhibitors can also be added to the chromatin sample.

DIRECT ChIP



INDIRECT ChIP



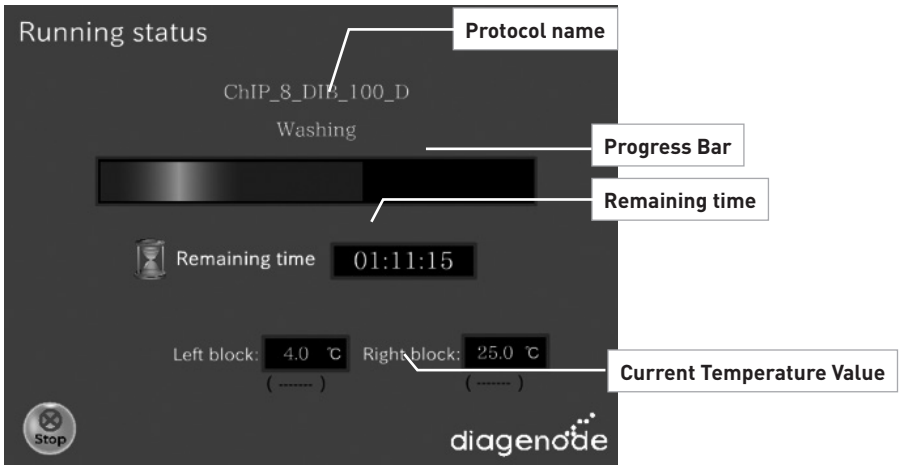
Screen – Set confirmation

After the user presses the "next" button in the "Layout information" screen, the "Set confirmation" screen appears.

At this point, user is expected to be ready to press RUN.

Buttons:

- The user presses the "Back" button. The user returns to the Layout information screen.
- The user presses the "Run" button. This is the expected action when user gets to this display after reviewing blocks. Runs the protocol.



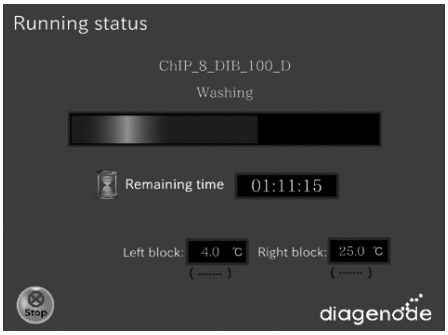
Screen – Running

After the user presses the "Run" button in the "Set confirmation" screen, the "Running" screen appears.

Buttons:

- The user presses the "Stop" button. Then screen shall be changes to "Stop Dialog".

Status screen is preferred as a progress bar that moves across the screen as the step progresses

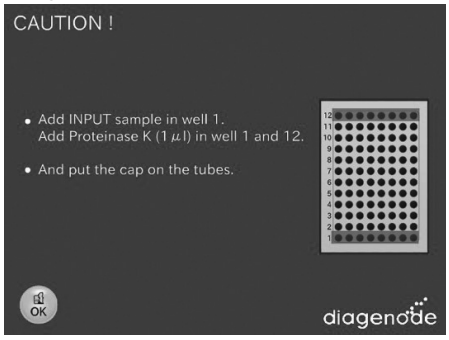


Screen – Running status

This screen gives informations about the current running step of the protocol.

The user can check through this screen the passed and remaining time of the experiment.

A. ChIP - DIB

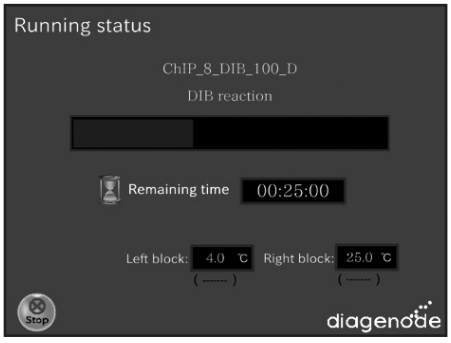


Screen – Elution

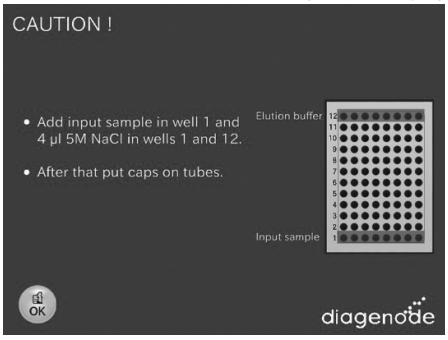
INPUT is defined as

a) 100µl protocol:
INPUT= 1µl diluted sheared chromatin
+ 99 µl DIB buffer

b) 200 µl protocol
INPUT= 2µl diluted sheared chromatin
+ 98 µl DIB Buffer



B. ChIP - IPure

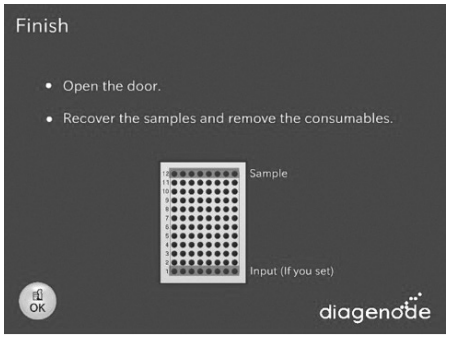

CAUTION !
• Add input sample in well 1 and
4 µl 5M NaCl in wells 1 and 12.
• After that put caps on tubes.
Elution buffer
Input sample
OK
diagenode

Screen – Elution

INPUT is defined as

a) 100µl protocol:
INPUT= 1µl diluted sheared chromatin
+ 95 µl Elution Buffer

b) 200 µl protocol
INPUT= 2µl diluted sheared chromatin
+ 94 µl Elution Buffer



Screen – Finish/End

When the protocol is complete, a window appears telling user the run is over. The screen behind this window should be the Startup screen. When OK is pressed, then the Startup screen appears and the user can immediately begin to remove their sample and prepare the next run.

At this point, user is expected to be ready to press RUN.

Buttons:

- The user presses the "OK" button. Then screen shall be changed to "[Categories Name] Protocol List".

Screen – Caution !

When the protocol finishes the user can return to the protocol list (screen **A.**) or warm the peltier block (screen **B.**) to eliminate possible condensation in the block.

Note 1: Please note that when isolating DNA with DIB buffer the DNA will be recovered in single strand conformation. When isolating DNA with Elution Buffer followed by reverse crosslinking, the DNA will be recovered in double strand conformation.

Note 2: RNase treatment by incubating the samples with RNase at 37°C during 30 minutes can be performed after the reverse crosslinking and it is recommended for ChIp-seq experiments. However, Diagenode does not provide RNase.

Note 3: DNA purification can be done using our simplified and validated Auto IPure kit from Diagenode. Alternatively, spin columns and Phenol-chloroform method can also be used.

SX-8G IP-STAR®

How to perform Automated ChIP in the SX-8G IP-Star®

How to perform Automated ChIP in the SX-8G IP-Star®

Chromatin preparation

STEP 1. Cell collection and DNA-protein crosslinking

1. Collect the cells by trypsinisation and wash two times with PBS.
2. Count the cells and resuspend them in PBS to obtain up to 10 million cells in 500 µl of PBS. Aliquot 500 µl of cell suspension in 1.5 ml tubes.
3. Add 13.5 µl of 36.5% formaldehyde per 500 µl sample. Mix by gentle vortexing and incubate for 8 minutes at room temperature to allow fixation to take place.
4. Stop the fixation by adding 57 µl of Glycine solution. Mix by gentle vortexing and incubate for 5 minutes at room temperature. Work on ice from this point onwards.
5. Centrifuge at 1,600 rpm (250 x g) for 5 minutes at 4°C and gently aspirate the supernatant without disturbing the cell pellet.
6. Wash the cells twice with 1 ml PBS.

STEP 2. Cell lysis and chromatin shearing

We recommend the use of Diagenode Bioruptor devices in combination with our shearing optimization kits (Table 3. p.10) for the preparation of the sheared chromatin.

STEP 3. Sheared chromatin and beads preparation

1. Start with sheared chromatin in Low, Medium or High SDS concentration depending on the cell type and the amount of cells sheared.
2. Dilute your sheared chromatin with ChIP Buffer H to reach a final concentration of 0.1-0.15% SDS. Dilute 10 times if using Chromatin shearing optimization kit - High SDS and 5 times if using Chromatin shearing optimization kit - Medium SDS.
3. Protease Inhibitors are provided in the kit. Add to the diluted sheared chromatin, protease inhibitors to a final concentration of 1X

Note 1: please mind in advance about the cell concentration during shearing process as the working IP volumes in the automated systems are 100 µl and 200 µl.

Note 2: In general, Diagenode does not recommend to preblocked Diagenode magnetic beads. BSA is however provided in this kit for customers that would like to proceed with preblocking or to add BSA in the immunoprecipitation reaction. For preblocking proceed as follow:

1. Pippette 100 ul of beads. Add 390 ul CHIP Buffer H and 10 ul BSA 5% (0.1% final concentration)
2. Incubate 60 minutes by rotation
3. Washed once with 100 ul of ChIP BUffer H.
4. Resuspend your beads in 100 ul ChIP Buffer H.
   10 ul of magnetic beads will bind up to 2.5 ug of Antibody.

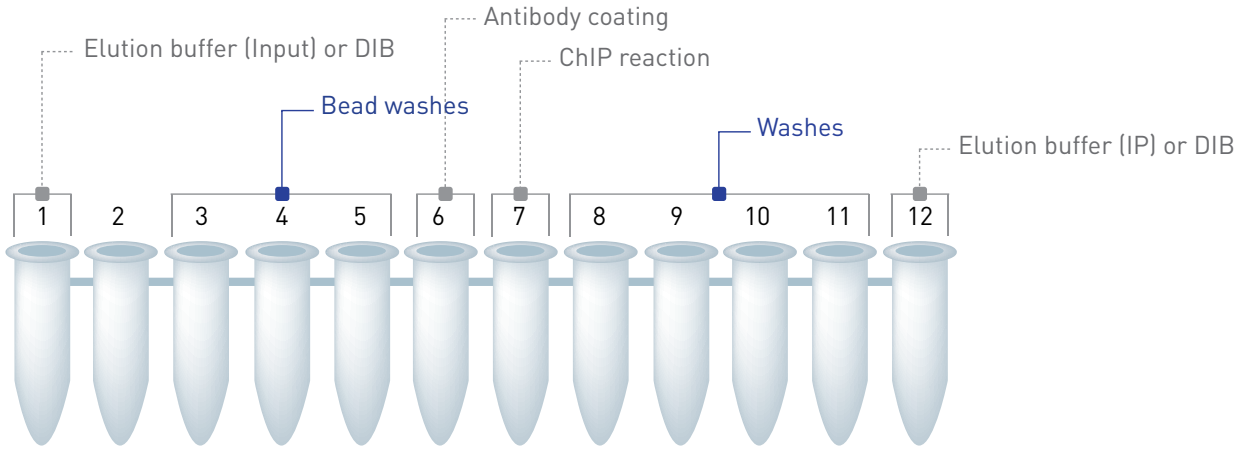
Dispense prepared reagents into the corresponding tubes (see picture below)

ChIP Direct Method (Ab coating)

With this method the antibody is first coated on the surface of the magnetic beads and after that the bound antibodies are added to the sheared chromatin.

2. Preparation Buffer + Ab

Antibody	x µl
ChIP Buffer H	100 - x



	IPURE			DIB		
Tube #	Description	100 µl protocol	200 µl protocol	Description	100 µl protocol	200 µl protocol
1	Elution buffer H + 5M NaCl	95 µl + 4 µl	94 µl + 4 µl	DIB	99 µl	98 µl
2	Empty	-	-	Empty	-	-
3	Magnetic beads*	10 µl	20-50 µl	Magnetic beads*	10 µl	20-50 µl
4	ChIP Buffer H	50 µl	100 µl	ChIP Buffer H	50 µl	100 µl
5	ChIP Buffer H	50 µl	100 µl	ChIP Buffer H	50 µl	100 µl
6	ChIP Buffer H + Ab	100 µl	100 µl	ChIP Buffer H + Ab	100 µl	100 µl
7	Sheared Chromatin Mix	100 µl	200 µl	Sheared Chromatin Mix	100 µl	200 µl
8	Wash Buffer H1	100 µl	150 µl	Wash Buffer H1	100 µl	150 µl
9	Wash Buffer H2	100 µl	150 µl	Wash Buffer H2	100 µl	150 µl
10	Wash Buffer H3	100 µl	150 µl	Wash Buffer H3	100 µl	150 µl
11	Wash Buffer H4	100 µl	150 µl	Wash Buffer H4	100 µl	150 µl
12	Elution buffer H + 5M NaCl	96 µl + 4 µl	96 µl + 4 µl	DIB	100 µl	100 µl

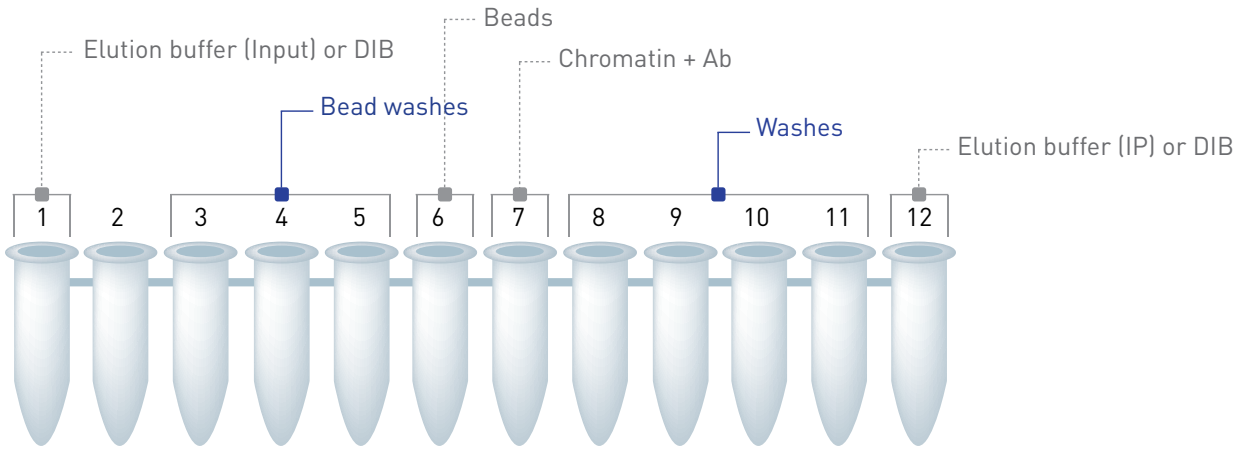
* This Auto Histone ChIP-seq kit has been optimized with Diagenode's high quality ChIP-grade antibodies and we use very low amounts of antibody per IP. The binding capacity of 10 µl of magnetic beads is ~3 µg of antibody. If you plan to use more than 3 µg of antibody per IP we recommend that the quantity of beads is adjusted accordingly. Please contact us for advice if required.

NOTE 1: If required, add 2 ul of BSA (5%) to the sheared chromatin mix when running ChIP-100 ul protocols or 4 ul of BSA (5%) when running ChIP-200 ul protocols

NOTE 2: If required, NaBu (HDAC inhibitior, 20mM final concentration) or other inhibitors can also be added to the sheared chromatin mix.

ChIP Indirect method (IP and beads incubation)

With this method the antibodies are incubated first with the sheared chromatin and after that the magnetic beads are added to the immunocomplex.



	IPURE			DIB		
Tube #	Description	100 µl protocol	200 µl protocol	Description	100 µl protocol	200 µl protocol
1	Elution buffer H + 5M NaCl	95 µl + 4 µl	94 µl + 4 µl	DIB	99 µl	98 µl
2	Empty	-	-	Empty	-	-
3	Magnetic beads*	10 µl	20-50 µl	Magnetic beads*	10 µl	20-50 µl
4	ChIP Buffer H	50 µl	100 µl	ChIP Buffer H	50 µl	100 µl
5	ChIP Buffer H	50 µl	100 µl	ChIP Buffer H	50 µl	100 µl
6	ChIP Buffer H	100 µl	100 µl	ChIP Buffer H	100 µl	100 µl
7	Sheared Chromatin Mix + Ab	100 µl	200 µl	Sheared Chromatin Mix + Ab	100 µl	200 µl
8	Wash Buffer H1	100 µl	150 µl	Wash Buffer H1	100 µl	150 µl
9	Wash Buffer H2	100 µl	150 µl	Wash Buffer H2	100 µl	150 µl
10	Wash Buffer H3	100 µl	150 µl	Wash Buffer H3	100 µl	150 µl
11	Wash Buffer H4	100 µl	150 µl	Wash Buffer H4	100 µl	150 µl
12	Elution buffer H + 5M NaCl	96 µl + 4 µl	96 µl + 4 µl	DIB	100 µl	100 µl

* This Auto Histone ChIP-seq kit has been optimized with Diagenode's high quality ChIP-grade antibodies and we use very low amounts of antibody per IP. The binding capacity of 10 µl of magnetic beads is ~3 µg of antibody. If you plan to use more than 3 µg of antibody per IP we recommend that the quantity of beads is adjusted accordingly. Please contact us for advice if required.

NOTE 1: If required, add 2 ul of BSA (5%) to the sheared chromatin mix when running ChIP-100 ul protocols or 4 ul of BSA (5%) when running ChIP-200 ul protocols

NOTE 2: If required, NaBu (HDAC inhibitior, 20mM final concentration) or other inhibitors can also be added to the sheared chromatin mix

Running protocol

Be sure that the computer connected to the SX-8G IP-Star never switches to the standby modus. (standby modus has to be inactivated). Standby of the computer will lead to the abort of the protocol.

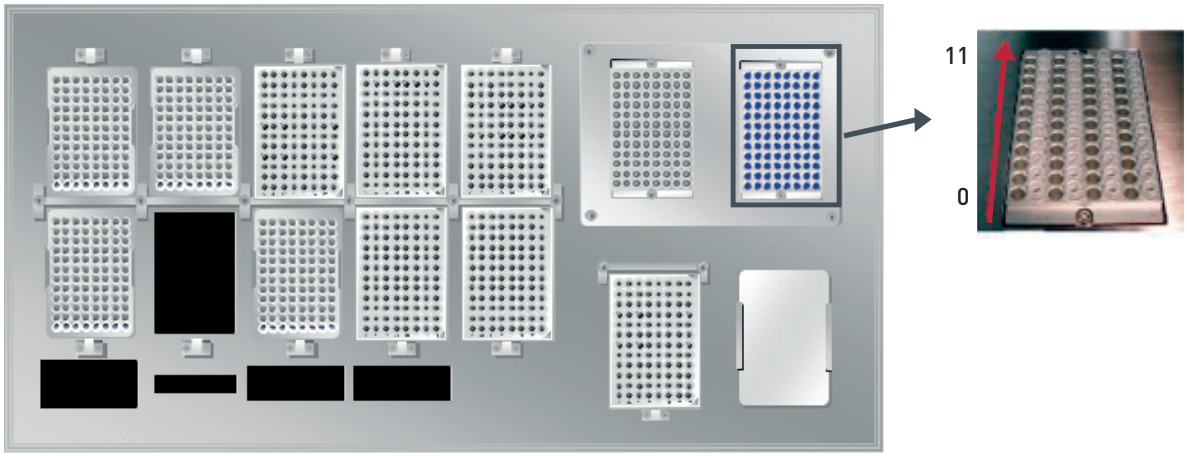
Table 3.

Protocol Name	ChIP DIB 8 protocol	ChIP IPure 8 protocol
Reagent Preparation*	1h	1h
Magnetic Bead Washes	30 min	30 min
Ab coating	Ab coating time	Ab coating time
Immunoselection	IP reaction time	IP reaction time
Washes and elution	30 min	1h
Add reagents	15 min	15 min
DNA isolation or reverse cross-linking	30 min (DNA isolation)	4h (reverse cross-linking)
DNA recovery	ss DNA	ds DNA

* Input required is sheared chromatin ready-to-ChIP

Note: Hands-on-work time is reduced to 1h45 min in both protocols!

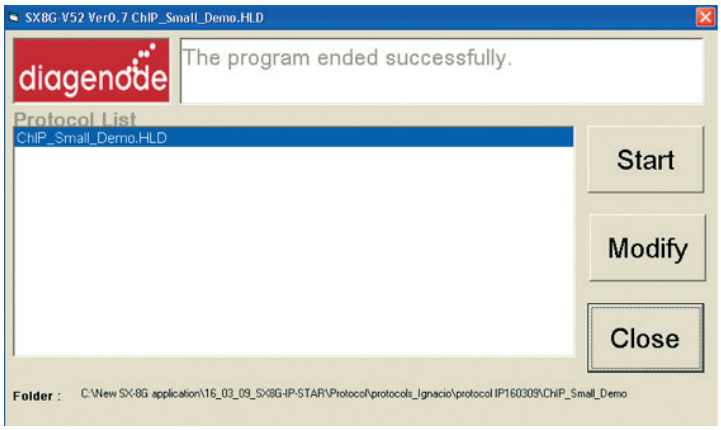
1. Switch on the SX-8G IP Star. The power switch is on the right side of the instrument.
2. Switch on the computer.
3. Start SX-8G V52 software through the following icon
4. Place the prepared tube strip on the right cooling / heating block of the workstation



5. Close the workstation door and lock it using the following icon

6. Press the following icon

Select the protocol of interest. Press start.



IMPORTANT NOTE:

If the ChIP protocols do not appear in the screen,

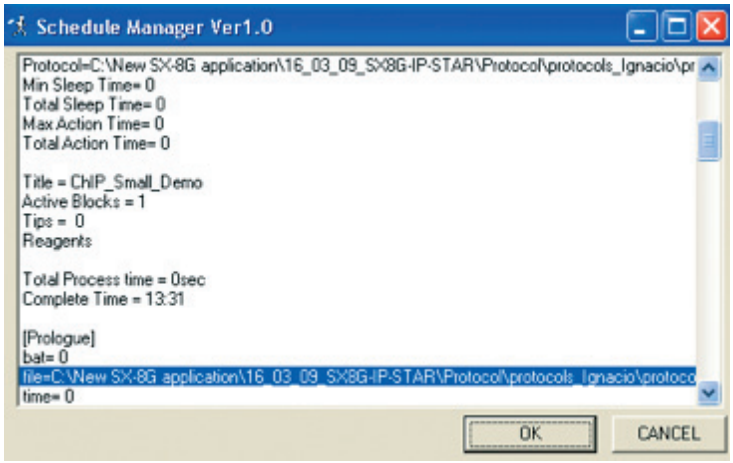
1. Open the SX-8V52 directory
2. Open Easy start ini file. Write the directory location of the protocols

   The Easy start ini file should contain the following information:

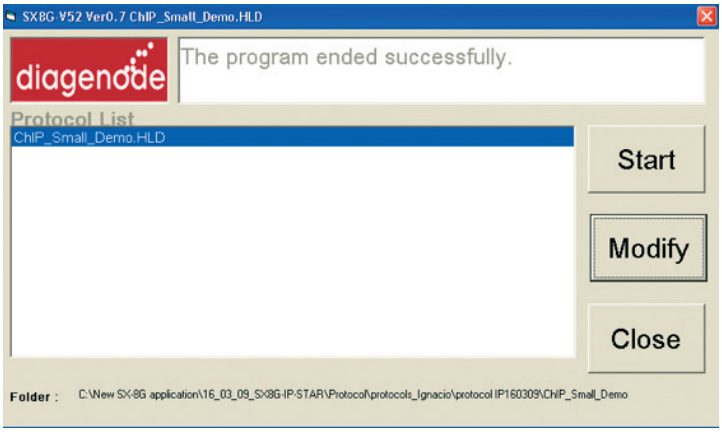
   *[**EASYSTARTSCREEN**]*
   *HoldFilePath = **C:\Documents and Settings\Desktop\New software protocols\ChIP\Ab Coating***
   for loading ChIP Direct protocols or
   *HoldFilePath = **C:\Documents and Settings\Desktop\New software protocols\ChIP\IP and beads incubation***
   for loading ChIP Indirect protocols
   In red it is indicated the directory location of the ChIP protocols.

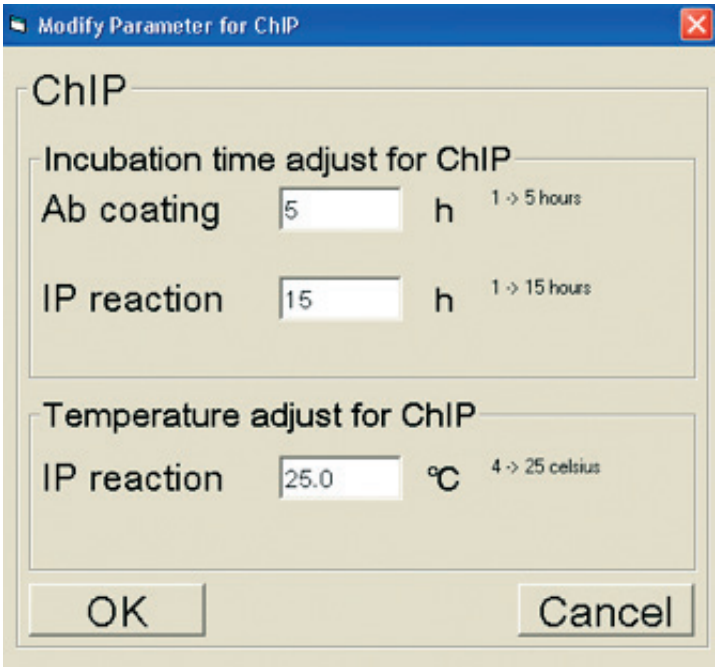
3. Start now SX-8G V52 software through SX-8G V52 exe.file
4. Press button for Easy Protocol Start screen and load the protocol of interest

Before starting the protocol a start confirmation window will appear. Press OK and the protocol will run.

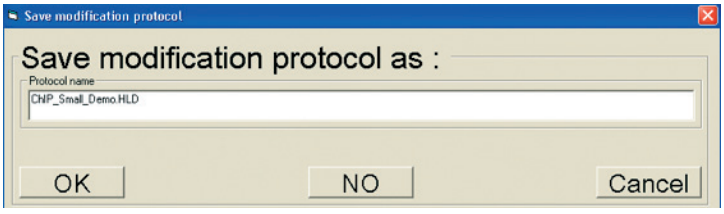


Alternatively, incubation time for antibody coating and temperature and incubation time for the IP reaction can be adjusted in an existing protocol by selecting the modify button. The modified protocol can also be saved as new protocol.

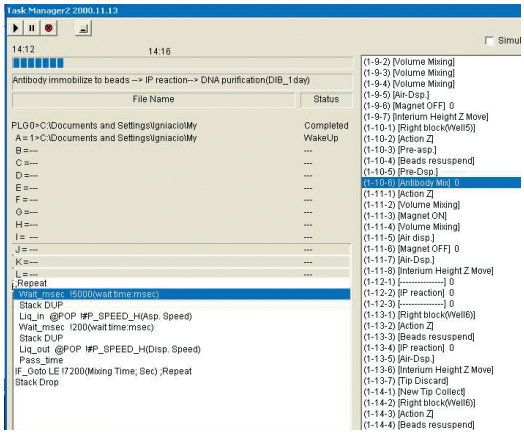




If running ChIP 16 protocol, setup half of the incubation time. It will incubate half of the time on each block but total time will be correct. (For instance, if you want 10h incubation, you have to setup 5h)



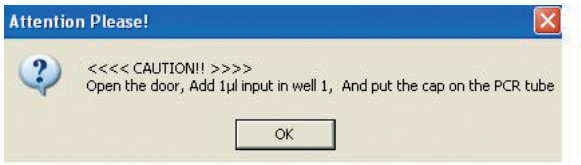
7. The program will run through the following steps: magnetic bead washes, IP and IP washes.



During protocol the next window will be displayed indicating the step that the protocol is processing.

8. Reverse crosslinking

After the IP washes the following window will be appear.

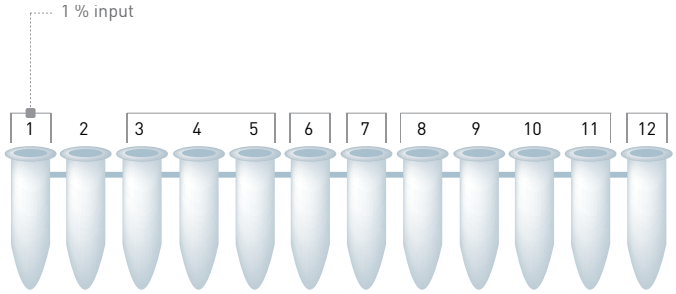


1. Add 1 % Input to well 1: 1 µl of input when using the 100 µl protocol ; 2 µl of input using the 200 µl protocol.

2. Close the tube strip with the corresponding caps.

3. Press OK.

4. Reverse crosslinking will be performed at 65°C for 4 hours or O.N.



Note: Optional. RNase treatment by incubating the samples with RNase at 37°C during 30 minutes can be performed after the reverse crosslinking. Diagenode does not provide RNase.

5. DNA purification can be done using our simplified and validated Auto IPure kit from Diagenode. Alternatively, spin columns and Phenol-chloroform method can also be used.

9. Shutting down the IP-Star®

1. Click on File and press End to close the software correctly.
2. Switch off the computer and its monitor.
3. Switch off the SX-8G IP-Star® Automated System (power switch on the right side).

Note: Ensure that the door is closed!

Quantitative PCR & Data Analysis

This last step consists in amplifying and analysing the IP'd DNA.

1. Prepare the **qPCR mix** using SYBR PCR Green master mix. **qPCR** cycles are given below.

   qPCR mix (total volume of 25 µl/reaction):

   1 µl of provided primer pair (stock: 5 µM each: reverse and forward)

   + 12.5 µl of master mix (e.g.: iQ SYBR Green supermix)

   + 5.0 µl of purified DNA sample and diluted purified input(s) (see above for input dilution)

   + 6.5 µl of water

qPCR cycles			
	Temperature	Time	Cycles
PCR Amplification	95°C	3 minutes	x1
	95°C	30 seconds	x40
	60°C	30 seconds	
	72°C	30 seconds	
Melting Curve	65°C and increment of 0.5°C per cycle	1 minute	x60

2. When the PCR is done, **analyse** the results. Some major advices are given below.

- **Your own primer design**
  - » Self-complementarity and secondary structure of the primers can be tested for primer design (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Annealing temperature of 60°C is recommended for qPCR primers.
  - » Short length of amplified DNA fragment (50 - 150 bp) facilitates the PCR efficiency and reduces potential problems in amplification of G/C-rich regions.
  - » Difference in melting temperature between forward and reverse primers should not exceed 2 to 3°C.
  - » G/C stretches at the 3' end of the primers should be avoided.

- **Advantages of the qPCR**
  - » qPCR or Real time PCR enable fast, quantitative and reliable results. Visit: http://www.gene-quantification.info/. The Gene Quantification page describes and summarises all technical aspects involved in quantitative gene expression analysis using real-time qPCR & qRT-PCR. It presents a lot of applications, chemistries, methods, algorithms, cyclers, kits, dyes, analysis methods, meetings, workshops, and services involved.

- **Validation of your primers**
  - » Test primer sets by in silico PCR: http://genome.cse.ucsc.edu/cgi-bin/hgPcr. Primers should amplify unique DNA products from the genome.
  - » Test every primer set in qPCR using 10 fold-serial dilutions of input DNA. Calculate amplification efficiency (AE) of primer set using the following by formula(5): AE= 10^(-1 / slope)
  - » The ideal amplification factor is 2. If it is not the case the qPCR reagents from different brand or new primers should be tested.
  - » qPCR products should also be run on a high resolution agarose gel since melting curve analysis in qPCR not always picks up primer dimmer or additional products.

- Data interpretation

  » The efficiency of chromatin immunoprecipitation of particular genomic locus can be calculated from qPCR data and reported as a percentage of starting material: % (ChIP/ Total input).

  » % (ChIP/ Total input)= 2^[(Ct(x%input) – log(x%)/log2) - Ct(ChIP)]x 100%

  » Here 2 is the amplification efficiency (AE) as calculated above(5); Ct (ChIP) and Ct (x%input) are threshold values obtained from exponential phase of qPCR for the IP'd DNA sample and input sample respectively; the compensatory factor (logx%/log2) is used to take into account the dilution 1:x of the input. The **recovery** is the % (ChIP/ Total input).

  Or

  » % input= AE^(Ctinput – CtChIP) x Fd x 100%

  » Here AE is amplification efficiency as calculated above (5); CtChIP and Ctinput are threshold values obtained from exponential phase of qPCR; Fd is a dilution factor of the input DNA to balance the difference in amounts of ChIP and input DNA taken for qPCR.

- Relative occupancy

  » Relative occupancy can be calculated as a ratio of specific signal over background.

  » Relative occupancy can be calculated as a ratio of specific signal over background:

  » Occupancy= % input (specific loci) / % input (background loci)

  Relative occupancy is then used as a measure of the protein association with a specific locus; it provides clues about specificity of ChIP. Highly specific ChIP can result in about 10 fold enrichment over background and some antibodies can reach up to 1000 fold. This value not only depends on the antibody but also on the target. ChIP result can be considered as reliable in case of significant values for both efficiency and specificity.

- Use of a **standard curve generated from fragmented genomic DNA**. A dilution series is made and qPCR is run on DNA with the primer one uses for ChIP. This will give the PCR efficiency. Most qPCR programs allow automatic calculation of the DNA quantity in the samples by comparing with the Ct and known quantities of DNA standards.

Results

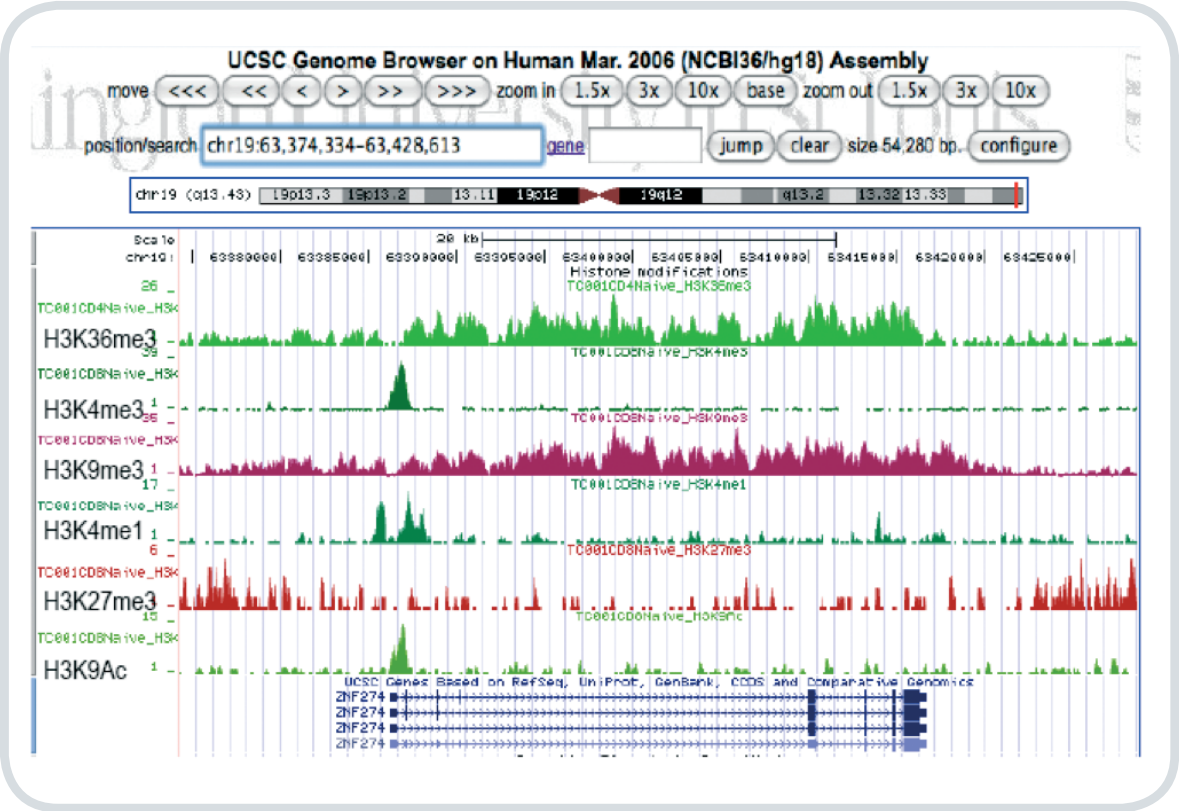


Figure. Auto ChIP-seq assays were performed on the SX8G IP-Star using primary human CD4 and CD8 T cells isolated by negative magnetic separation of peripheral blood mononuclear cells. Each Auto-ChIP sample was performed using Diagenode's Auto Histone ChIP-seq kit reagents and contained 1 ug of input chromatin. Illumina Genome Analyzer libraries were prepared and the samples were sequenced at the DNA Technologies Core at UC Davis.

Aditional Protocols

Sheared chromatin analysis

This protocol refers to the Diagenode's Elution module (Cat. No. mc-magme-002) that can be ordered separately.

Reagents not supplied

- RNase cocktail (e.g. Ambion, AM 2286 A)
- Phenol/chloroform/isoamyl alcohol (25:24:1)
- Chloroform/isoamyl alcohol (24 : 1)
- 100% Ethanol
- 70% Ethanol
- Agarose and TAE buffer
- TE

1. Take an aliquot of 100 µl of sheared chromatin and spin the chromatin at 14,000 x g (13,000 rpm) for 10 min at 4°C. Transfer the supernatant to a new tube for chromatin analysis.

   A minimum of 60 000 cells is needed to be vizualized onto agarose gel. If each 100 µl of sheared chromatin correspond to 10 000 cells, then perform 6 reactions in parallel and pool the DNA pellets obtained at Step 14 during respuspension in TE.

2. Prepare RNase cocktail dilution (e.g. Ambion, AM 2286 A: dilute 1 µl of cocktail in 150 µl of water).

3. Add 2 µl of diluted RNase cocktail to the chromatin.

4. Incubate 1h at 37°C.

5. Prepare the Complete Elution Buffer by mixing thoroughly Buffer D, E and F (Elution module) as follow :

Reagents	Volume
Buffer D	96 µl
Buffer E	10 µl
Buffer F	4 µl
Total volume	**110 µl**

6. Add 100 µl of the Complete Elution Buffer to each chromatin sample.

7. Mix thoroughly and incubate samples at 65°C for 4 hours (or overnight).

8. Extract DNA once with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). Incubate the samples at RT for 10 minutes on a rotating wheel.

9. Centrifuge for 2 minutes at 14,000 xg (13,000 rpm) at RT. Transfer the top aqueous phase into a new 1.5 ml tube.

10. Add 1 volume of chloroform/isoamyl alcohol (24:1). Incubate the samples at RT for 10 minutes on a rotating wheel.

11. Centrifuge for 2 minutes at 14,000 x g (13,000 rpm) at RT. Transfer the top aqueous phase into a new 1.5 ml tube.

12. Precipitate the DNA by adding 40 µl of meDNA precipitant, 5 µl of meDNA co-precipitant and 1 ml 100% cold ethanol to the sample. Incubate at -80°C for 30 minutes.

13. Centrifuge for 25 minutes at 14,000 x g (13,000 rpm) at 4°C. Carefully remove the supernatant and add 500 µl of ice-cold 70 % ethanol to the pellet.

14. Centrifuge for 10 minutes at 14,000 x g (13,000 rpm) at 4°C. Carefully remove the supernatant, leave tubes opened for 30 minutes at RT to evaporate the remaining ethanol.

15. Resuspend the pellet in 10 µl of TE. That corresponds to the purified DNA from the sheared chromatin. Several DNA pellets can be pooled at this step to have DNA corresponding to a minimum of 60 000 cells in 10 µl of TE.

16. Run samples (10 µl of DNA + 2 µl of 6x loading dye) in a 1.5% agarose gel along with DNA size marker to visualise shearing efficiency.

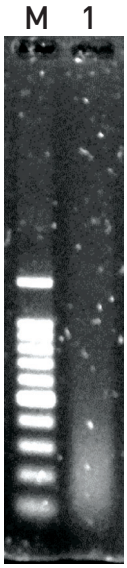


Figure 3: Hela cells were fixed with 1% formaldehyde (for 10 minutes at RT). Cell lysis are performed using the Lysis Buffer tL1 of the Diagenode True MicroChIP kit. Samples corresponding to 10 000 cells are sheared during 5 rounds of 5 cycles of 30 seconds "ON" / 30 seconds "OFF" with the Bioruptor® Plus combined with the Bioruptor® Water cooler (Cat No. BioAcc-cool) at HIGH power setting (position H). For optimal results, samples are vortexed before and after performing 5 sonication cycles, followed by a short centrifugation at 4°C. 10 µl of DNA (equivalent to 60 000 cells) are analysed on a 1.5% agarose gel. (lane 1; lane M: 100 bp DNA Molecular Weight Marker)

Troubleshooting Guide

Error Cause	Remedy
SX-8G IP-Star cannot be switched on	SX-8G IP-Star is not receiving power. Check that the power cord is connected to the workstation and to the wall power outlet.
Computer cannot be switched on	Computer is not receiving power. Check that the power cord is connected to the computer and to the wall power outlet.
SX-8G IP-Star shows no movement when a protocol is started	SX-8G IP-Star is not switched on. Check that the SX-8G IP-Star is switched on.
SX-8G IP-Star shows abnormal movement when a protocol is started	The pipettor head may have lost its home position. In the Software, select "Manual Operation/Home". After confirming that the pipettor head moves to the home position, run the protocol again.
Aspirated liquid drips from the disposable tips	Dripping is acceptable when ethanol is being handled. For other liquids: air is leaking from the syringe pumps. Grease or replace the O-rings. If the problem persists, contact DIAGENODE Technical Services.

Technical Assistance & Ordering Information

At DIAGENODE we pride ourselves on the quality and availability of our technical support. Our Technical Services Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of DIAGENODE products. If you have any questions, or experience any difficulties regarding the SX-8G IP-Star or DIAGENODE products in general, do not hesitate to contact us.

DIAGENODE customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at DIAGENODE. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information call the DIAGENODE Technical Service Department or contact your local distributor.

Diagenode s.a. BELGIUM | EUROPE
LIEGE SCIENCE PARK
Rue Bois Saint-Jean, 3
4102 Seraing - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
techsupport@diagenode.com
orders@diagenode.com

Diagenode Inc. USA | NORTH AMERICA
400 Morris Avenue, Suite #101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
techsupport.na@diagenode.com
orders.na@diagenode.com

For a complete listing of Diagenode's international distributors, visit:
http://www.diagenode.com/en/company/distributors.php
For the rest of the world, please contact Diagenode s.a.

diagenode

Innovating Epigenetic Solutions

Ordering information

Description	Cat. No. (NEW)	Cat. No. (OLD)	Format
SX-8G IP-Star® Compact	B03000002	UH-002-0001	1 unit
Auto True MicroChIP kit	C01010140	/	16 rxns
Auto True MicroChIP & MicroPlex Library Prep Package	C01010141	/	16 ChIP rxns & 12 library prep rxns
MicroPlex Library Preparation kit x12	C05010010	AB-004-0012	12 rxns
Auto Histone ChIP-seq kit protein A x16	C01010020	AB-Auto02-A016	16 rxns
Auto Histone ChIP-seq kit protein A x100	C01010022	AB-Auto02-A100	100 rxns
Auto Histone ChIP-seq kit prowwtein G x16	C01010021	AB-Auto02-G016	16 rxns
Auto Histone ChIP-seq kit protein G x100	C01010023	AB-Auto02-G100	100 rxns
Auto Transcription ChIP kit protein A x16	C01010030	AB-Auto03-A016	16 rxns
Auto Transcription ChIP kit protein A x100	C01010032	AB-Auto03-A100	100 rxns
Auto Transcription ChIP kit protein G x16	C01010031	AB-Auto03-G016	16 rxns
Auto Transcription ChIP kit protein G x100	C01010033	AB-Auto03-G100	100 rxns
Auto ChIP kit protein A x100	C01010011	AB-Auto01-A100	100 rxns
Auto ChIP kit protein G x100	C01010013	AB-Auto01-G100	100 rxns
Auto MeDIP kit x16	C02010011	AF-Auto01-0016	16 rxns
Auto MeDIP kit x100	C02010012	AF-Auto01-0100	100 rxns
Auto hMeDIP kit x16	C02010033	AF-Auto02-0016	16 rxns
Auto MethylCap x48	C02020011	AF-Auto01-0048	48 rxns
Auto IPure kit	C03010010	AL-Auto01-0100	100 rxns

Visit us at one of Diagenode's demo sites or discover our Automated Systems by performing some assays with the help of our R&D and Technical Department.

www.diagenode.com

DIAGENODE HEADQUARTERS

Diagenode s.a. BELGIUM | EUROPE
LIEGE SCIENCE PARK
Rue Bois Saint-Jean, 3
4102 Seraing - Belgium
Tel: +32 4 364 20 50 | Fax: +32 4 364 20 51
orders@diagenode.com
info@diagenode.com

Diagenode Inc. USA | NORTH AMERICA
400 Morris Avenue, Suite #101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680 | Fax: +1 862 209-4681
orders.na@diagenode.com
info.na@diagenode.com

For a complete listing of Diagenode's international distributors visit:
www.diagenode.com/en/company/distributors.php
For rest of the world, please contact Diagenode s.a.

© 2014 Diagenode, Inc. All rights reserved. The content of this document cannot be reproduced without prior permission of the authors. Bioruptor and IP-Star are registered trademarks of Diagenode.